**Title**

# New Insights into No-Go, Non-Stop and Nonsense-mediated mRNA Decay Complexes

**Short Title**

mRNA Decay Complexes

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**Abstract**

Eukaryotes possess a variety of translational control mechanisms which function in the surveillance of mRNAs, discriminating between normal and aberrant translation elongation and termination, triggering mRNA decay. The three major evolutionarily conserved eukaryotic pathways are No-Go, Non-Stop and Nonsense-Mediated mRNA Decay. Recent findings suggest that stalling of the ribosome, due to mRNA secondary structure or translation into poly(A)-stretches, leads to ribosome collisions which are detected by No-Go/Non-Stop mRNA decay factors. Subsequent ribosome ubiquitination at the interface of two collided ribosomes is considered the signal for mRNA decay. Similarly, translation termination at a premature stop codon is slower than normal, leading to recruitment and activation of nonsense-mediated mRNA decay factors, including SMG1-8-9. Here, we detail new insights into the molecular mechanisms of these pathways.

**Keywords:**

Translational control, No-Go mRNA Decay, Non-Stop mRNA Decay, Nonsense-Mediated mRNA Decay, ribosome-associated quality control, ribosome collisions

**Highlights:**

- collided ribosomes form a unique disomal interface and are a hallmark of slow/stalled translation
- ribosome ubiquitination is triggered by ribosome collisions targeting proteins at the disome interface
- ribosome ubiquitination is linked to No-Go/ Non-Stop mRNA decay and to ribosome-associated quality control
- SMG1-8-9 structures and biochemistry indicate that GTP binding/hydrolysis by SMG9 regulates SMG1 activity
- SMG8 conformational rearrangements could limit access to SMG1's kinase active site regulating UPF1 phosphorylation, a key step in NMD

## Introduction

Messenger RNAs (**mRNA**) and their protein products have highly regulated lifespans which are particularly important in “timed” cellular processes such as the cell cycle and differentiation. However, as a by-product of their biogenesis, many mRNAs contain defects either in their sequence, processing, or folding. Due to this, cells utilise pathways which survey the pool of mRNA to scrutinise their quality in a variety of ways [1]. Classically, detection of a mRNA without a 3’-poly-adenylated (**poly(A)**) tail will then target this mRNA to degradation. However, other defects in the mRNA sequence or structure are more difficult to detect. Here, cells rely on active translation and translational control pathways, including No-Go Decay (**NGD**), Non-Stop Decay (**NSD**) and Nonsense-Mediated mRNA Decay (**NMD**) in eukaryotes [2,3] (**Figure 1A**). These pathways scrutinise the activity of translation, detecting when elongation isn’t progressing normally (NGD/ NSD) or when translation termination occurs early (NMD) at a premature termination codon (**PTC**). Upon detection, these pathways trigger abortion of translation, recycling of the translation machinery, and degradation of the mRNAs. In contrast, in normal translation, initiation and elongation proceed uninhibited until a stop codon is encountered. Release of the nascent polypeptide is catalysed by release factors eRF1 and eRF3a [4] (**Figure 1B**). Subsequent recycling of ribosomal 40S and 60S subunits is mediated by ribosome recycling factors Rli1 (ABCE1 in mammals) (**Figure 1B**), eIF1, eIF1A, eIF3, and eIF3j [5].

In addition to mRNA decay, stalled ribosomes trigger the ribosome-associated quality control pathway (**RQC**) which marks the encoded nascent polypeptide products with polyubiquitin for subsequent degradation by the proteasome [6]. Substrates for RQC are large ribosomal subunits (**60S**) containing a peptidyl-tRNA in the active centre and associated peptide in the ribosomal tunnel. These substrates are generated by an RQC-trigger complex, comprising Slh1 RNA helicase-family protein, ubiquitin-binding protein Cue3, and yKR023 [7,8]. Together with Rli1/ABCE1 and Dom34 (Pelota in mammals) (**Figure 1A**), these proteins dissociate stalled 80S ribosomes into 40S and 60S subunits which are associated with mRNA and peptidyl-tRNA respectively. 60S-peptidyl-tRNA complexes are recognised by Rqc2 (NEMF in mammals) and by E3 ubiquitin ligase Ltn1 (listerin in mammals). Rqc2/NEMF attaches a random sequence of C-terminal alanine and threonine residues (a ‘CAT’-tail) to the nascent polypeptide in a reaction that does not require mRNA or the small ribosomal subunit 40S [9]. Ltn1/ listerin modifies lysines of the nascent chain in the immediate vicinity of the exit of the ribosomal tunnel with ubiquitin. By addition of the CAT tail to the nascent polypeptide, lysines hidden in the ribosomal tunnel can become accessible for ubiquitination by Ltn1/listerin. Subsequently, the ubiquitinated nascent chain is released by Vms1 (ANKZF1 in mammals) and degraded by the proteasome [10,11].

NGD, NSD and NMD are conserved from yeast to humans, but are specific to eukaryotes. Prokaryotes and archaea have evolved very different mRNA quality control pathways to recognise and degrade faulty mRNAs and their protein products, and there is virtually no conservation regarding the factors involved [12,13]. This review will focus on recent developments in the NGD, NSD and NMD pathways with an emphasis on recent mechanistic insights provided by cutting-edge biochemistry and structural biology.

## No-Go and Non-Stop Decay Target Stalled Elongating Ribosomes

NGD and NSD pathways were found to be activated by ribosome stalling during elongation [2,3]. Stalling can occur due to poly(A) read-through, large mRNA secondary structure, stretches of rare codons resulting in an unoccupied ribosomal A-site, starvation and deficiency conditions, and/or mRNA truncations. As the ribosome is unable to proceed, the ribosome-nascent chain complex becomes trapped, which is detected and handled by the NGD/NSD machinery consisting of Dom34/Pelota and Hbs1 (**Figure1A**). Subsequent mRNA cleavage by an endonuclease recruited to the stalled ribosome by an unknown mechanism supports degradation by the exosome and Xrn1 exonuclease. Dom34/Pelota and Hsb1 are structural homologues of eRF1 and eRF3a, respectively [14]. However, Dom34/Pelota does not detect stop codons. Together with Hbs1, Dom34/Pelota binds to an empty ribosomal A-site. Subsequent GTP hydrolysis by Hbs1 leads to accommodation of Dom34/Pelota in the 60S peptidyl-transferase centre. Unlike eRF1 which possesses a conserved GGQ motif mediating peptidyl-tRNA hydrolysis, Dom34/Pelota lack this motif and have three or four basic residues preceded by a proline instead **(Figure 1C)**. Therefore, the peptide is still attached to tRNA and 60S after ribosome dissociation and needs to be released by the RQC machinery [6]. Dom34/Pelota-Hbs1 recruit Rli1/ABCE1 and promote the dissociation of the stalled ribosomes.

## Poly(A)-Mediated Translation Stalling

The most frequent stalling event is caused by translation into the mRNA poly(A) tail due to lack of an in-frame stop codon [15,16]. In this case, translation is slowed due to interaction of the nascent poly(Lysine) (encoded by poly(A)) within the ribosomal exit tunnel [17,18] which triggers NSD. In *S. cerevisiae,* Ski7, a Hsb1 paralogue, recognises ribosomes stalled on the poly(A) tail. In higher eukaryotes Ski7 is not present, and NSD relies on Dom34/Pelota-Hsb1 complexes which dissociate the stalled ribosomes with the help of Rli1/ABCE1 [19,20]. In this respect, NGD and NSD utilise the same factors, differing only in their substrate mRNA and associated ribosomal stalling mechanism. Recently, poly(A) stalling was shown to involve both suboptimal tRNA conformations in the 60S peptidyl-transferase centre and an unproductive mRNA conformation in the 40S decoding centre [21••]. A 2.8 Å cryo-EM structure of a poly(A)-stalled mammalian ribosome was determined, showing that mRNA in the decoding centre adopts a single-stranded helical form, incompatible with tRNA-eEF1A-GTP binding due to steric clashes [21••]. This helical conformation is specific to poly(A) tracts, while not occurring efficiently in poly(AAG) tracts (also encoding poly(Lys)) [22]. In the 60S peptidyl-transferase centre, the C-terminal lysine of the peptidyl-tRNA was positioned with its side chain pointing at the A-site while the backbone carbonyl-group pointed away from the A-site. Such a conformation results in repulsive charge interaction with Lys-tRNA in the A-site, impeding proper Lys-tRNA accommodation and peptidyl transfer. In fact, the reactive groups in the peptidyl-transferase centre are positioned ~1.5 Å farther apart than in normal elongating ribosomes. Using varying lengths of mixed lysine tracts encoded by AAG and AAA, it was shown that both a poly(Lys)-tRNA in the peptidyl-transferase centre and the ribosomal tunnel and a poly(A) tract in the decoding centre are required for efficient stalling and subsequent NSD.

**NGD and NSD Activation requires Ribosome Ubiquitination**

Recently, NGD and NSD were found to require site-specific ribosome ubiquitination for recognition of stalled ribosomes [23,24•]. In mammals, the E3 ubiquitin ligase ZNF598 targets 40S ribosomal proteins eS10 and to a lesser degree uS10. Similarly, Hel2 (yeast orthologue of ZNF598) ubiquitinates the ribosomal proteins uS10 and uS3 and assists the RQC pathway in dissociation of stalled ribosomes [25••,26]. uS3 is dispensable for endonucleolytic cleavage, indicating that uS10 is the functional ubiquitination target for NGD. In fact, NGD is triggered by ribosomal stalling *and* subsequent ribosomal collision events of the stalled ribosome with the following translating ribosome(s) [27] (**Figure 2A**). Endonucleolytic mRNA cleavage which initiates mRNA decay depends on the density of ribosomes on the mRNA, with more collisions resulting in more cuts. Biochemically, collided ribosomes were identified and discriminated from normal mono-ribosomes and polysomes by micrococcal nuclease treatment, resulting in larger mRNA fragments being protected from digestion by collided ribosomes [27].

**Collided Ribosomes Present a Unique Platform for Recognition by NGD /NSD factors**

E3 ubiquitin ligase ZNF598 recognises a unique surface formed by two collided ribosomes, named a disome [25••]. Using a mammalian *in vitro* translation system, ZNF598-ribosome binding and ZNF598-associated ubiquitin transfer was found to be specifically enriched in collided ribosomal complexes. In contrast, ribosome ubiquitination was absent in the monosome fractions and collision-free polysomes supporting the conclusion that collided ribosomes are preferred substrates for ZNF598 [25••]. The cryo-EM structure of a mammalian collided disome [25••] revealed that the leading stalled ribosome had an empty E-site, peptidyl-tRNA in the P-site, and an inactive eRF1(AAQ) mutant in the A-site complexed with ABCE1 (**Figure 2B**). The following collided ribosome was in a rotated state with hybrid A/P- and P/E-site tRNAs. Interestingly, no density for elongation factor eEF2 was present in the collided ribosome despite this conformation being a target for eEF2, suggesting that this interaction is sterically blocked in the disome. The collided ribosomes interact via two interfaces. First, ribosomal proteins surrounding the mRNA exit channel (eS1, uS11, eS26, and eS28) of the leading ribosome contact elements near the mRNA entrance channel of the following ribosome (uS4 and helix 16 of 18S rRNA). Together, this positions the mRNA channels of leading and collided ribosomes near one another, explaining mRNA protection against micrococcal nuclease (**Figure 2B**). Second, RACK1 of the leading ribosome contacts uS3, uS10, and eS10 of the colliding ribosome; eS10 is the primary target for ubiquitination by ZNF598 in mammals, and uS10 is Hel2's ubiquitination target in yeast (**Figure 2B**). [23,24•,25]. As ZNF598's ubiquitination sites on the following collided ribosome were very close to the disome interface (**Figure 2B**), while the same sites were >50 Å away on the leading ribosome, ZNF598 was suggested to bind the disome interface and ubiquitinate eS10 on the collided ribosome, rationalising why collided ribosomal complexes are ubiquitinated, while monosomes are not [25••].

The yeast cryo-EM structure of a collided disome stalled by poly(Arginine) codons revealed a similar disome conformation and tRNA occupancies in A-, P-, and E-sites [28••]. uS10 was found in close

proximity to the disome interface providing a unique structural surface required for recognition by Hel2 E3 ubiquitin ligase. In the structure, the mRNA was confined in a narrow channel formed by ribosomal proteins and rRNA such that the mRNA in this disome unit is inaccessible to micrococcal nuclease. Biochemical data further supported that disomes present the preferred substrate for endonucleolytic cleavage which occurred at sites within the disome unit, one at the P-site of the leading ribosome and three others within the following collided ribosome [28••]. In a Hel2-truncation context, endonuclease cleavage no longer occurs within the collided disome, but alternatively occurs in downstream ribosomes (beyond the disome) suggestive of an alternative pathway [28••]. This pathway, believed to be independent of RQC, relies on Not4 mono-ubiquitination on ribosomal protein eS7.

Both collided disome structures lacked extra density near the ubiquitination sites corresponding to either ZNF598 or Hel2 [25••,28••]. Thus, further work is needed to elucidate the binding site of these E3 ubiquitin ligases. In yeast, NGD is functionally coupled to RQC for the leading disomal unit, in that ubiquitination of uS10 by Hel2 is required for RQC function [28••]. It is unclear how uS10-ubiquitinated ribosomes activate RQC. The RNA helicase Slh1 (and ASCC3 in mammals) is implicated in this RQC triggering step [8,28••], but the mechanistic details remain enigmatic.

**Cue2 is the Endonuclease Involved in NGD and NSD**

The identity of the endonuclease involved in yeast NGD/NSD was recently discovered [29]. Cue2 (N4BP2 in mammals) was shown to be necessary and sufficient for mRNA cleavage on stalled, collided ribosomes and does so within the A-site of the following colliding ribosome reliant on the partial displacement of A/P-tRNA's which were demonstrated in the determined disomal structures [25••,28••]. Homology modelling suggests that Cue2 binds in the 40S A-site such that conserved catalytic residues are positioned near the mRNA [29]. Cue2 has two putative ubiquitin-binding domains which may recognise the Hel2-mediated ubiquitination on collided ribosomes, potentially supporting recruitment of Cue2 and activation. Further, Slh1 is suggested to direct mRNAs to alternative degradation pathways, thus modulating the relative contribution of Cue2 in mRNA decay in NGD/NSD [29].

**Nonsense-mediated mRNA Decay**

NMD recognises and degrades mRNAs containing a PTC introduced by mutation, transcriptional errors, or aberrant splicing [30,31]. Recognition and elimination of PTC-containing mRNAs by NMD is intimately linked to genetic disease and cancer. Stop codon mutations account for ~20% of all genetic diseases caused by single-base pair mutations [32,33]. NMD of such PTC-containing mRNAs can suppress or enhance the effects of human genetic disease, depending on whether the encoded truncated protein is partially active, like in Duchenne muscular dystrophy, or toxic to the cell, like in some forms of beta-thalassemia [34]. In cancer, oncogenes were found to often contain missense mutations while tumour suppressor genes frequently have stop codon mutations triggering NMD and protein insufficiency [32]. Despite its medical importance, the role of individual NMD factors in this process is still poorly understood. The most conserved NMD factors are up-frameshift proteins (**UPF)**

UPF1, UPF2 and UPF3B. The human NMD machinery additionally is comprised of suppressors with morphogenetic effects on genitalia (**SMG**) including SMGs 1, 5, 6, 7, 8, and 9. SMG1 forms a complex with SMG8 and SMG9 which downregulate SMG1's kinase activity [35].

NMD factors are suggested to be recruited to the mRNA due to slowed translation termination. While most normal stop codons are positioned in the last exon, PTC-containing mRNAs are often characterised by the presence of downstream exon-junction complexes (**EJC**) [36]. Importantly, active translation is a critical prerequisite to discriminate a normal stop codon from a PTC [37]. At a normal stop codon, poly(A)-binding protein (**PABP**), bound to the poly(A) tail in proximity to the stop codon, stimulates the recruitment of eRF1 and eRF3a supporting peptide release (**Figure 1B**) [38] and contributes to ribosome recycling and re-initiation of translation [39]. At a PTC, translation termination is slowed down by the absence of PABP stimulation [38,40]. In the absence of PAPB, the EJC-associated NMD factor UPF3B impedes translation termination and supports dissociation of ribosomes after termination [41]. Interaction of UPF3B with the ribosome and release factors, which would occur in the vicinity of the ribosomal A-site, interferes with stop codon recognition and peptide release at a PTC [41]. Importantly, UPF1 seems to have no function in translation termination at a PTC, despite being bound in the downstream 3'-UTR of the mRNA and directly to the ribosome [41,42]. Subsequent recruitment of UPF2 to UPF1 and UPF3B by a currently unknown mechanism supports activation of SMG1-8-9 and of UPF1 [43] (**Figure 1A**).

A key activation step in NMD is the recruitment and activation of the SMG1-8-9 kinase complex to phosphorylate UPF1, likely in the context of the PTC-stalled ribosome. SMG1 is suggested to be activated by interaction with UPF2 and UPF3B [44]. UPF1 phosphorylation by SMG1 activates UPF1's ATPase and helicase functions [35,44,45], and is believed to enable remodelling of NMD complexes. Phospho-UPF1 recruits the endonuclease SMG6 to cleave the mRNA close to the PTC-stalled ribosome [46]. Furthermore, phospho-UPF1 binds SMG5-7 which recruits additional components supporting the removal of the 5'-cap of the mRNA, deadenylation of the mRNA, and triggering the exonucleolytic mRNA decay by Xrn1 and the exosome [47].

## Cryo-EM Structures of SMG1 and SMG1-8-9

Structural detail of the SMG1-8-9 complex was, until recently, limited to a crystal structure of the core complex of SMG8-9 [48] and low-resolution cryo-EM maps of SMG1 alone and in complex with SMG8-9 and UPF1 [49,50]. Recently, two high-resolution cryo-EM structures of the SMG1-8-9 complex were reported [51••,52••]. SMG1 is a member of the phosphatidyl inositol kinase-like kinase (**PIKK**) family and structurally similar to mammalian Target of Rapamycin kinase (**mTOR**) [53]. SMG1 comprises a C-terminal kinase domain with a highly conserved fold amongst PIKK family members (**Figure 3 A-B**). The N-terminus is formed by long N-terminal Huntingtin, Elongation factor-3, protein phosphatase 2A, and TOR1 (**HEAT**) repeats. SMG1's HEAT domain forms a C-shaped arch, followed by a head domain which possesses the catalytic kinase component referred to as FATKIN [54]. The FATKIN is composed of 3 parallel middle HEAT repeats (**M-HEAT**) and a kinase domain (**KD**) built of two lobes, resembling

typical serine/threonine kinase domains, which are packed against the focal-adhesion-targeting (**FAT**) and FAT C-terminal (**FATC**) domains [55] (**Figure 3A**). Following the KD, and prior to FATC, is an insertion of 1,200 residues which is unique to SMG1 relative to other PIKK members, but not resolved in the cryo-EM structures.

**SMG8 and SMG9 Inhibit SMG1 Kinase Activity**

Determination of SMG8-9's structure in SMG1-8-9 relied on a *C. elegans* SMG8-9 crystal structure [48]. SMG8-9 forms a pseudo-symmetric dimer mediated by hydrophobic surfaces. SMG8 and SMG9 both contain a globular core domain, referred to as a G-fold, which is related to the dynamin-like GTPase family [48]. SMG9 binds SMG1 by inserting a 15 amino acid loop into a groove between SMG1's HEAT repeats 10 and 11 (**Figure 3B**). This interaction is further aided by a stretch of SMG9 residues wedged between the head and arch domains of SMG1. SMG8 binds SMG1 further away from the head, by inserting a helical stretch into a hydrophobic groove between HEAT repeats 8 and 10. SMG9 is oriented to face SMG1's kinase active site in a manner similar to that seen in mTOR binding by its regulatory factor RHEB [53]. In the absence of SMG8-9, SMG1 displays an overall similar fold as when in complex with SMG8-9 (RMSD 2.3 Å), but with major localised differences in the N-terminal HEAT repeat which adopts a more curved conformation in the complex with up to a 12 Å shift of the HEAT repeats positioning them further away from the head domain.

Given SMG8-9's predicted GTPase-folds, the SMG8-9 crystal was supplemented with GDP which was found only in SMG9 [48]. In the SMG1-8-9 cryo-EM structure, density was detected in SMG9's nucleotide-binding site [51••]. GTP was modelled into this density with a coordinated magnesium ion in classical GTPase fashion [56]. When comparing SMG8-9's conformations in the GTP- and GDP-bound forms, the positions of SMG8 and SMG9 are substantially different. Up to 10 Å shifts in a previously unassigned region of SMG8 are evident (**Figure 3 C-D**), positioning this domain over the catalytic pocket of SMG1's KD, covering the substrate entry groove. Therefore, this region was named the kinase-inhibitory domain (**KID**) [51••]. Deletion of SMG8's KID had no impact on its ability to bind to SMG1, but resulted in hyper-activation of UPF1 phosphorylation relative to wildtype activity, indicating a regulatory function for this domain. From this, a model was suggested by which GTP hydrolysis by SMG9 results in a conformational rearrangement of SMG8-9 allowing UPF1 access into the catalytic site of SMG1 and phosphorylation [51••]. Interestingly, in the second study, ESI-MS and reverse phase ion-pair HPLC was used to identify the identity of the nucleotide co-purified with SMG1-8-9 [52••], indicating that ATP, not GTP, is bound to SMG9.

Both cryo-EM structures did not reveal the position of C-insertion domain of SMG1 (**Figure 3A**), suggesting that this domain is highly flexible. Like deletion of the KID, deletion of the C-insertion domain resulted in SMG1 hyper-activation [49], indicating that the C-insertion is also involved in regulation of kinase activity. Taken together, the identity of the nucleotide bound to SMG9, SMG9's possible GTPase activity, and the roles of SMG8-9 and the C-insertion domain in regulation of SMG1's activity require further investigation. Additionally, given the key role of SMG1 phosphorylation of UPF1 in triggering

NMD, it is essential to understand UPF1's specific binding to SMG1-8-9 and the activation of SMG1-8-9 by UPF2 and UPF3 in the context of the stalled PTC-containing ribosome.

## Concluding Remarks

Recent work has made significant progress in our mechanistic understanding of NGD and NSD. Collided ribosomes have been identified as a prerequisite for the detection of ribosomal stalling and activation of NGD and NSD. Ubiquitination of the collided ribosomes plays a key role in triggering nucleolytic cleavage of the mRNA bound to the collided ribosomes. However, a detailed understanding of the chain of events is lacking. Further, ubiquitin transfer to disomal ribosomal proteins was shown to activate NGD *and* RQC pathways, coupling mRNA decay and nascent polypeptide degradation [28••], but a more detailed mechanistic understanding of the interplay is needed.

In comparison, progress in determining the potential roles of collided ribosomes and ubiquitination involved in NMD mechanisms remains limited. The fact that one of the collided ribosome structures was solved with a stalled terminating ribosome [25••] suggests that ribosome collisions occur during termination at a PTC as well. However, it is currently unclear if ribosomal collision events and ribosome ubiquitination are important for triggering NMD. Similarly, the fate of the C-terminally truncated nascent polypeptide is unclear. Interestingly, UPF1 was shown to have E3 ubiquitin ligase activity [57], but there is no direct evidence for ubiquitination of the nascent polypeptide in the context of NMD. Finally, despite many attempts, there is an utter lack of structures of NMD factors bound to PTC-stalled ribosomes which are required to advance our understanding of the assembly of the NMD machinery as well as recruitment and activation of the SMG1-8-9 complex.

## Conflict of Interest

The authors declare no conflict of interest.


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The structure of a yeast disome is determined by cryo-EM supported by biochemical data demonstrating that Hel2 E3 ubiquitin ligase specifically targets disomal units and ubiquitinates ribosomal protein uS10 which resides at the disome interface. Hel2 and Slh1/Rqt2 are required for NGD and RQC function. Nuclease cleavage occurs within the disomal unit.

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The first high-resolution structures of SMG1 and the SMG1-8-9 complex are determined by cryo-EM suggesting that SMG8 has a C-terminal kinase inhibition domain which occludes the kinase active site and may rely on SMG9's interconversion between GTP- and GDP-bound states for conformational rearrangement to regulate SMG1's kinase activity.

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**Figure Legends**

**Figure 1: Translational quality control. A)** No-Go, Non-Stop, and Nonsense-Mediated mRNA decay stalled ribosomal complexes. The ribosome (PDBID:5LZT [58]), composed of 60S (light blue) and 40S (light yellow) subunits, is translating along a stylised representation of a 5'-capped and 3'-poly(A) tailed mRNA (black) synthesising a nascent polypeptide (yellow). Ribosomal E-, P-, and A-sites are indicated (white). tRNAs (PDBID:5LZT [58]) are bound in the E- (peach) and P- (orange) sites; pathway-specific factors are bound in the A-site. Top: In No-Go decay, Pelota (red) is complexed with Hbs1 (light green) in the A-site (PDBID:5LZX [58]). Pelota-Hbs1 recognise elongating ribosomes stalled prior to a stop codon (red STOP sign), e.g., due to a stable mRNA secondary structure element. GTP hydrolysis by Hbs1 leads to accommodation of Pelota into the peptidyl-transferase centre and recruitment of ribosome-recycling factor ABCE1 (dark green) for ribosome dissociation steps. Nucleases are recruited through an unknown mechanism. Middle: Non-Stop decay relies on the same factors as No-Go decay, but differs in its mRNA substrate. Read-through into the 3'-poly(A) tail (due to absence of a stop codon) slows and stalls elongating ribosomes triggering NSD steps involving Pelota, Hbs1, and ABCE1. Bottom: Nonsense-mediated mRNA decay is represented by a ribosome stalled at a PTC (white STOP sign), with the EJC (MAGOH, RBM8A/Y14, eIF4A3, and CASC3 in light brown (PDBID:3EX7 [59])) between the PTC and its native stop codon (red STOP sign). The presence of NMD factor UFP3B (green) and the absence of PABP (blue) stimulation slows down recruitment of eRF1 (magenta) and eRF3a (bright pink) and subsequent peptide release. NMD factors UPF2 (orange) and UPF3B are suggested to activate the SMG1 (purple)-SMG8 (green)-SMG9 (dark blue) complex for UPF1 (red) phosphorylation which triggers recruitment of mRNA decay factors. **B)** Translation termination at a normal stop codon. Top: The translating ribosome has encountered a stop codon in proximity of PABP which stimulates recruitment of release factors eRF1 and eRF3a and recognition of the stop codon in the ribosomal A-site (PDBID:5LZT [58]). Middle: Hydrolysis of GTP by eRF3a allows dissociation of eRF3a and accommodation of eRF1 (PDBID:5LZU [58]), leading to peptidyl-tRNA hydrolysis. Bottom: Recruitment of ribosome-recycling factor ABCE1 (dark green (PDBID:5LL6 [60])) supports dissociation of ribosomal subunits. **C)** Three structures are shown illustrating the structural similarity of tRNA (orange), eRF1 (magenta), and Pelota (Dom34, red) with individual functional motifs indicated.

**Figure 2: Ribosomal collision events and ribosome ubiquitination trigger mRNA quality control. A)** Top: Cartoon representation of multiple elongating ribosomes. The leading ribosomes slows or stalls (red lines) due to mRNA secondary structure, rare codons, or mRNA truncations (dark red stars). Bottom: The following translating ribosome collides with the leading stalled ribosome forming a disomal unit. **B)** Structure of the disomal unit with stalled (60S: dark blue, 40S: dark yellow) and collided (60S: light blue, 40S: light yellow) ribosomes (PDBIDs:6HCJ [25••], 6HCF [25••], and 6I7O [28••]). The disome interface is supported primarily by interactions between 40S subunits of leading and collided ribosomes centring around the mRNA (black) exit and entry channels. The inlay shows the unique interface which forms between the two collided ribosomes including ZNF598 and Hel2 ubiquitination sites eS10 (magenta) and uS10 (green) respectively.

**Figure 3: SMG1-8-9 complex activation is required to trigger UPF1 phosphorylation and mRNA decay. A)** Cartoon diagrams of SMG1 (purple and pink), SMG8 (green), and SMG9 (blue) indicating domains and residue numbers for the domain boundaries. The KID of SMG8 lacks a border and boundaries as this predicted domain's density was present, but not resolved in the cryo-EM structure [51••]. **B)** The structures of SMG1-8-9 and of SMG1 alone [51••] (PDBID:6L54 [51••]) are shown in two views (90° rotation). Domains of SMG1, SMG8 and SMG9 are labeled. **C)** The structure of SMG1's head region (purple) in complex with GTP-bound SMG-9 (light blue) and SMG-8 (light green) (PDBID: 6L54 [51••]) is overlaid with aligned GDP-bound (PDBID:5NKK [48]) SMG-9 (dark blue) and SMG-8 (dark green). The relative shift in SMG-8 regions is proposed to contribute to altering the position of the KID relative to SMG1's active site regulating substrate access into the active site. **D)** Cartoon representation of the predicted positional shift of the KID domain in GTP- and GDP-bound contexts illustrating KID's proposed role in occluding access of UPF1 to the kinase active site of SMG1 in the GTP-bound conformation.

Figure

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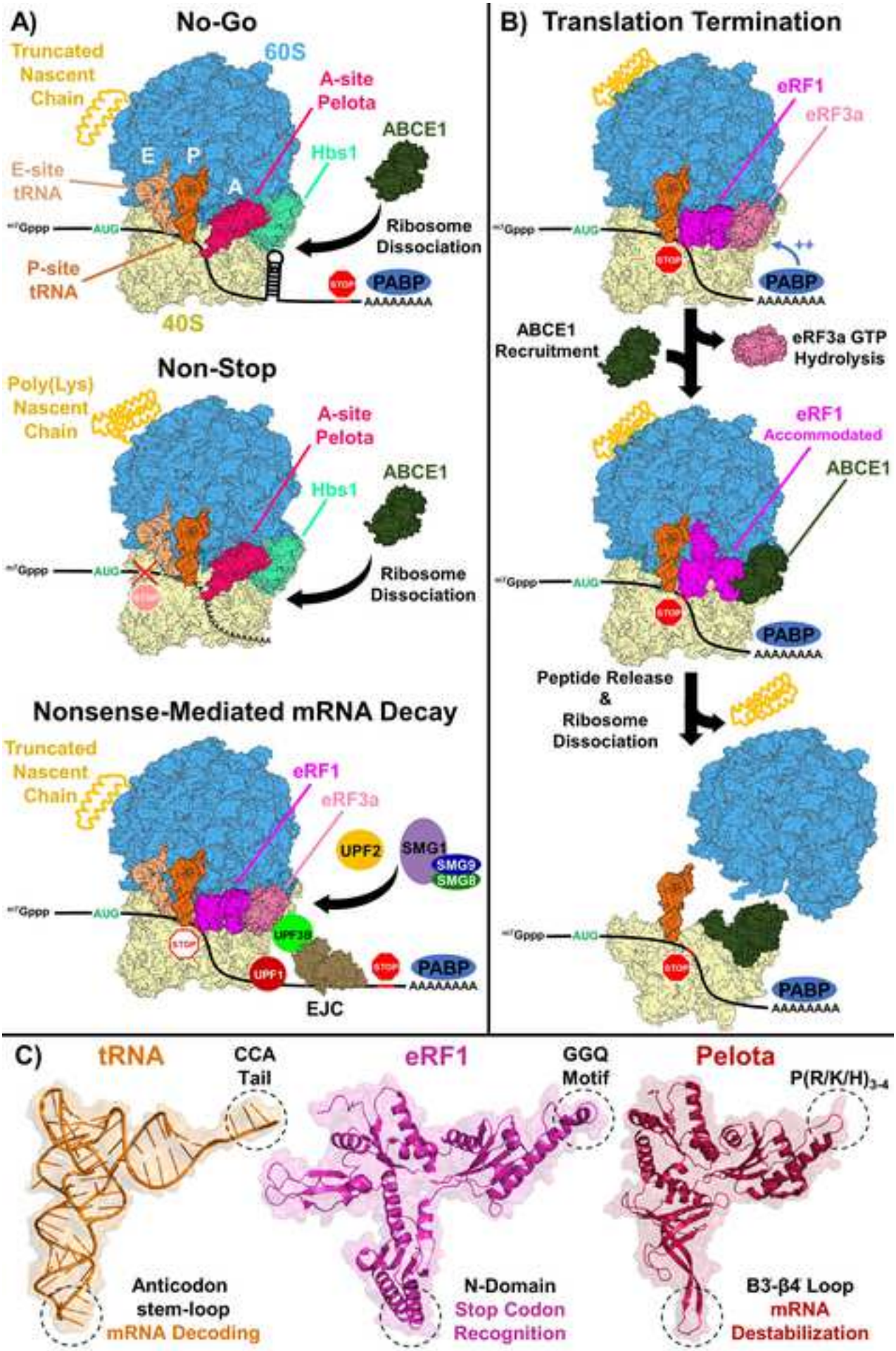
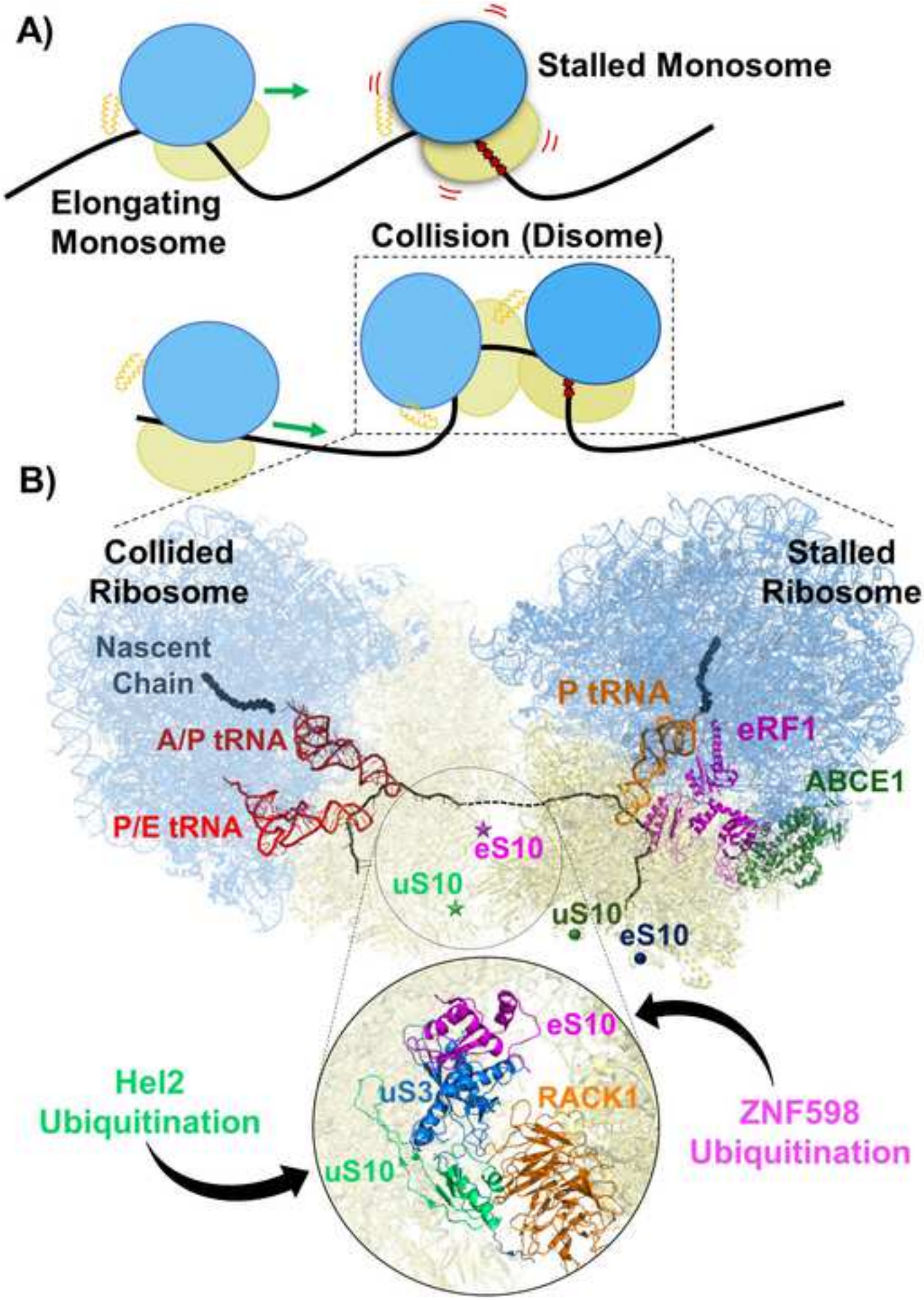


Figure





Figure

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